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The effect of severe eccentric exercise-induced muscle damage on plasma elastase, glutamine and zinc concentrations

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Abstract The aim of this study was to determine if severe exercise-induced muscle damage alters the plasma concentrations of glutamine and zinc. Changes in plasma concentrations of glutamine, zinc and polymorphonuclear elastase (an index of phagocytic cell activation) were examined for up to 10 days following eccentric exercise of the knee extensors of one leg in eight untrained subjects. The exercise bout consisted of 20 repetitions of electrically stimulated eccentric muscle actions on an isokinetic dynamometer. Subjects experienced severe muscle soreness and large increases in plasma creatine kinase activity indicative of muscle fibre damage. Peak soreness occurred at 2 days post-exercise and peak creatine kinase activity [21714 (6416) $U \cdot l^{-1}$, mean (SEM)] occurred at 3 days post-exercise ($P < 0.01$ compared with pre-exercise). Plasma elastase concentration was increased at 3 days post-exercise compared with pre-exercise ($P < 0.05$), and is presumably indicative of ongoing phagocytic leucocyte infiltration and activation in the damaged muscles. There were no significant changes in plasma zinc and glutamine concentrations in the days following eccentric exercise. We conclude that exercise-induced muscle damage does not produce changes in plasma glutamine or zinc concentrations despite evidence of phagocytic neutrophil activation.

Key words Muscle damage · Zinc · Glutamine · Immune function

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Introduction

Eccentric-exercise-induced muscle damage is associated with increases in the plasma levels of muscle-specific enzymes indicative of myofibre damage, leucocyte infiltration of the affected muscles, muscle soreness, swelling and weakness (Jones et al. 1986). Other forms of tissue injury, including elective surgery, burns, trauma and infection, are associated with falls in the plasma concentration of glutamine, which may be detrimental to immune function because glutamine is required for the optimal functioning of leucocytes (Ardawi and Newsholme 1985; Parry-Billings et al. 1990a). The overtraining syndrome is associated with a reduction in plasma glutamine levels (Parry-Billings et al. 1992; Rowbottom et al. 1996) and this may be at least partly responsible for the immunosuppression apparent in this condition (Newsholme 1994). Skeletal muscle is the major source of glutamine production in the body. One of the most common symptoms reported in overtrained athletes is muscle soreness (Eichner 1995), and we hypothesized that muscle damage may be responsible for falls in the plasma glutamine concentration. Theoretically, this could arise via decreased synthesis/release of glutamine from damaged skeletal muscle and/or increased glutamine uptake from the blood by other tissues involved in the associated inflammatory acute phase response to injury including activated leucocytes and the liver. Thus, one of the aims of the present study was to determine if falls in plasma glutamine concentration occur in the days following an acute bout of eccentric exercise. We also wished to establish if changes in the plasma concentration of polymorphonuclear elastase (an enzyme released from activated neutrophils) occur, as this might give an indication of the time course of leucocyte infiltration into muscle following exercise involving eccentric muscle actions.

Zinc is a trace mineral important in maintaining normal immunological function (Cordova and Alvarez-Mon 1995) and is a cofactor of many enzymes involved

in energy metabolism. Acute prolonged exercise has been shown to transiently increase the plasma zinc concentration and exercise may increase zinc requirements because zinc loss from the body in urine and sweat is enhanced as a result of prolonged exercise (Couzy et al. 1990). It has been suggested (Brouns 1993) that the increased zinc excretion may be related to elevated plasma zinc levels arising from muscle fibre damage induced by mechanical forces during exercise. Plasma zinc concentration increased significantly over several weeks in cyclists during the Tour de France (Saris et al. 1989), but a study by Nosaka and Clarkson (1992) did not confirm effects of exercise-induced muscle damage on plasma zinc levels. However, the latter study examined changes in plasma zinc after voluntary eccentric exercise of a small muscle group (elbow flexors) and some of the subjects did not exhibit large increases in plasma creatine kinase activity. Thus, another aim of the present study was to determine if increases in plasma zinc concentration occur in the days following an acute bout of eccentric exercise involving a large muscle group (knee extensors). We used electrically evoked muscle activation to ensure a substantial degree of muscle damage in all subjects.

Methods

Subjects and protocol

Following approval from the Wolverhampton University Ethics Committee, eight untrained, but recreationally active, subjects (six females and two males), aged 22 (1.2) years, body mass 62.9 (3.6) kg, height 1.64 (0.04) m [mean (SEM)] performed a bout of 20 electrically stimulated eccentric muscle actions of the knee extensors of one leg on an isokinetic dynamometer (Kin-com, Chattecx, Chattanooga,Tenn, USA) at an angular velocity of 1.05 rad $\cdot$ s^{-1} through a range of motion of 1.75 rad. Electrostimulation at 50 Hz was delivered via large felt-covered copper electrodes soaked in water and placed on the skin surface over the muscle. The stimulation voltage was set prior to the bout and was that which was sufficient to produce at least 40% of maximal voluntary contraction force in an isometric contraction at a knee joint angle of 1.57 rad. The stimulation commenced approximately 1 s prior to movement of the leg to allow muscle force to increase to a maximum prior to movement. Resting venous blood samples were taken by venepuncture from an antecubital vein before the exercise, then again on days 1, 3, 7 and 10 after exercise. All blood samples were taken at the same time of day and in the post-absorptive state (at least 4 h after the last meal). Blood was obtained by needle and syringe and was immediately decanted into heparinised vials (LIP Equipment, Shipley, Yorkshire, UK). After centrifugation at 1500 *g* for 10 min, plasma was stored in 0.5 ml aliquots at −70°C prior to analysis.

Biochemical analysis

Plasma creatine kinase activity was determined at 30°C using an enzymatic kit (No. 47-10, Sigma, Poole, UK). Plasma zinc concentration was determined using a colorimetric kit (Wako, Neuss, Germany). Plasma elastase concentration was determined using an ELISA kit (Merck, Lutterworth, UK) and plasma glutamine concentration was measured by enzymatic spectrophotometric determination of the ammonia concentration before and after treatment of the plasma with glutaminase (Walsh et al. 1997).

Rating of muscle soreness

Subjective soreness in the knee extensors of the exercised leg was assessed on each day for up to 10 days post-exercise. Subjects were required to palpate six sites on the anterior muscles of the upper leg. Soreness elicited was recorded using a questionnaire incorporating a visual- analogue scale from 1 (normal) to 10 (very, very sore) for each of the six sites. The resultant values from each questionnaire were summed for the criterion score (minimum = 6, maximum = 60).

Statistical analysis

Non-parametric data (soreness ratings) were analysed by the Kruskal-Wallis test with post-hoc Wilcoxon signed rank tests. Parametric data were examined using ANOVA with a repeated-measures design, with post-hoc *t*-tests. Correlations were analysed using Spearman's rank order formula. An alpha level of 0.05 was used to determine statistical significance.

Results

All subjects experienced muscle soreness in the days following the eccentric exercise bout and muscle soreness was greatest on day 2 post-exercise (Table 1). Large increases in plasma creatine kinase activity were observed, peaking at day 3 (Table 1), indicating that a significant degree of muscle damage had occurred in all subjects.

No change in plasma zinc concentration was observed on days 1, 3 ,7 or 10 following exercise compared with pre-exercise (Table 2). Similarly, no significant change in plasma glutamine concentration occurred in the days following the bout of eccentric muscle actions (Table 2).

No change in plasma elastase concentration was observed on day 1 [mean (SEM) 30.2 (4.7) ng $\cdot$ ml^{-1}] compared with pre-exercise [28.0 (2.6) ng $\cdot$ ml^{-1}, $P > 0.05$]. Increases in plasma elastase concentration were observed later (Table 2), with peak values in different individuals occurring on days 3,7 or 10 following exercise [peak elastase concentration was 43.5 (3.5) $\mu g \cdot l^{-1}$, $P < 0.05$ compared with pre-exercise]. The subject who exhibited by far the largest peak plasma creatine kinase activity (61,212 U $\cdot$ l^{-1}) had a large,

Table 1 Changes in mean (SEM) plasma creatine kinase (CK) activity after electrically evoked eccentric muscle actions. Also shown are median [range] criterion scores for ratings of muscle soreness

	Plasma CK (U $\cdot$ l^{-1})	Muscle soreness rating
Pre-exercise	302 (127)	6 [6–6]
1 day post-exercise	5440 (2571)*	22 [13–42]**
2 days post-exercise	Not measured	31 [15–42]**
3 days post-exercise	21714 (6416)**	25 [13–30]**
4 days post-exercise	Not measured	14 [7–24]*
7 days post-exercise	3462 (909)**	8 [6–16]
10 days post-exercise	737 (263)	6 [6–8]

Significantly different from pre-exercise: *$P < 0.05$, **$P < 0.01$

Table 2 Changes in mean (SEM) plasma zinc, glutamine and elastase concentrations after electrically evoked eccentric mucle actions

	Zinc ($\mu mol \cdot l^{-1}$)	Glutamine ($\mu mol \cdot l^{-1}$)	Elastase ($ng \cdot ml^{-1}$)
Pre-exercise	13.1 (2.0)	549 (47)	28.0 (2.6)
1 day post-exercise	12.4 (1.1)	549 (44)	30.2 (4.7)
3 days post-exercise	13.0 (1.0)	589 (30)	38.1 (3.5)*
7 days post-exercise	11.5 (0.9)	598 (44)	37.9 (5.0)
10 days post-exercise	11.8 (1.4)	594 (44)	38.7 (5.1)

Significantly different from pre-exercise: $*P < 0.05$

sustained increase in plasma elastase concentration, and the two lowest creatine kinase responders (peak values of 1246 and 13,562 $U \cdot l^{-1}$), appeared to have the smallest elastase response. However, there was no significant correlation between peak plasma creatine kinase activity and peak plasma elastase concentration for the group as a whole ($r = 0.43$; $P > 0.05$).

Discussion

The rise in the plasma elastase concentration in the days following eccentric-exercise-induced muscle damage presumably reflects an increased activity of phagocytic neutrophils. Increases in plasma elastase concentration have been reported to occur during prolonged exercise (Dufaux and Order 1989) but such effects are transient: plasma elastase concentration returns to pre-exercise levels within 24 h. There is histological evidence of leucocyte infiltration of muscle after eccentric exercise in humans (Jones et al. 1986), and most of the infiltrating cells appear to be phagocytic macrophages and neutrophils. These are associated with necrosis of damaged muscle fibres and during phagocytosis neutrophils release digestive enzymes including elastase, some of which is likely to be released into the extracellular fluid and subsequently enter the circulation, together with some muscle proteins including creatine kinase. Another reason for the plasma elastase concentration rising could be an increase in the numbers of circulating neutrophils, but other studies have reported that no rises in circulating neutrophils occur in the days following eccentric exercise (e.g Gleeson et al. 1995). The time course of the rise in plasma elastase concentration after eccentric exercise agrees with biopsy studies which have observed a progressive increase in the leucocyte infiltration of affected muscles over several days after a bout of damaging exercise (e.g. Hikida et al. 1983; Jones et al. 1986); in these studies peak leucocyte numbers in biopsied muscle samples were reported to occur 7–10 days post-exercise. However, in comparison to systemic infections in which the plasma elastase concentration can rise approximately 20-fold (Speer et al. 1987), increases in plasma elastase observed in the present study were relatively small. This may be because only relatively few muscle fibres are damaged after eccentric actions (Jones et al. 1986). Hence, the extent of tissue injury and the associated inflammatory responses may be less after exercise-induced muscle damage compared with other forms of tissue trauma and infection.

The present study confirms that exercise-induced muscle damage does not affect the plasma concentration of zinc. Increases in plasma zinc concentration that have been reported to occur after prolonged running and cycling may be due to haemoconcentration and intravascular haemolysis, since the concentration of zinc in erythrocytes is about ten times that in plasma (Cousins 1989). The acute phase response to stress, including injury and endotoxin challenge, involves depression of circulating zinc (and iron) levels (Klasing 1984). There is some evidence that these effects are the result of granulocyte (neutrophil) degranulation (Goldblum et al. 1987). However, in the present study, despite evidence of neutrophil degranulation 3 days following eccentric-exercise-induced muscle damage, there was no change in plasma zinc at this time. It is, of course, possible that an increased release of zinc from damaged muscle fibres could be cancelled out by increased release of zinc-chelating proteins from activated neutrophils.

We also conclude that exercise-induced muscle damage does not affect the plasma concentration of glutamine. Other forms of tissue injury including burns (Parry-Billings et al. 1990b) and elective surgery (Wernerman and Vinnars 1987) are associated with falls in the plasma (and muscle) glutamine concentration, so it seems somewhat surprising that exercise-induced damage of a large muscle group does not have a similar effect. Whether this is due to a relatively small number of muscle fibres being affected by eccentric exercise or some other reason is presently impossible to say. Certainly, responses to exercise-induced muscle damage differ in a number of respects from other forms of inflammatory injury; for example, increases in circulating leucocytes are not observed in the days after eccentric exercise, whereas a large sustained neutrophilia follows elective surgery. Increases in plasma cytokines are also less marked after eccentric exercise-induced muscle damage (Bruunsgaard et al. 1997) compared with other forms of trauma. It seems likely that the minority of severely damaged muscle fibres following eccentric exercise is not sufficient to provoke an inflammatory response capable of inducing profound alterations in zinc and glutamine metabolism that can be detected at the plasma level. Muscle soreness is a common symptom among overtrained athletes (Eichner 1995), who have been reported to exhibit low concentrations of plasma glutamine (Parry-Billings et al. 1992; Rowbottom et al. 1996). From results of the present study we tentatively suggest that eccentric-exercise-induced muscle damage is not likely to be a cause of the low plasma glutamine concentrations observed in overtrained individuals.

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